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Antioxidant Capacity and Phenolic Content of Sweet Rowanberries

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Sweet rowanberry cultivars adapted to northern climates have been developed from rowanberries (*Sorbus aucuparia* L.) and hybrids of rowanberry with *Malus*, *Pyrus*, *Aronia*, or *Mespilus*. The rowanberries studied here (cvs. Burka, Dessertnaja, Eliit, Granatnaja, Kubovaja, Rosina, Rubinovaja, Titan, and Zholtaja) have high antioxidant and phenolic contents. The phenolic content varied between 550 and 1014 mg/100 g of fresh weight in sweet rowanberries, whereas 846 and 717 mg were found in the well-characterized bilberry and lingonberry, respectively. Anthocyanins (6−80 mg) were mainly found from berries of hybrid cultivars. Of the other phenolics, chlorogenic (29−160 mg) and neochlorogenic (34−104 mg) acids constituted the major fraction in all rowanberries, the concentrations almost equaling those present in coffee. Antioxidant capacities of rowanberries were high, as measured with FRAP (61−105 μmol of Fe^{2+}/g) and DPPH (21.3−9.7 g/g DPPH) methods. Principal component analysis was able to separate the cultivars of different origin into clusters on the basis of their phenolic profiles.

KEYWORDS: Sweet rowanberry; *Sorbus aucuparia*; rowanberry; phenolic compound; chlorogenic acid; antioxidant; FRAP; DPPH

INTRODUCTION

High consumption of plant phenolics in the daily diet has been found to provide protection against cardiovascular diseases and cancer, although contradictory studies have also been reported (*1*−*3*). Bioactive properties of phenolic compounds have been explored in numerous in vitro studies, which show their ability to suppress, for example, oxidation of low-density lipoprotein (*4*), platelet aggregation (*4*), growth of tumor cells (*5*), and inflammation reactions (*6*).

Numerous plant species have been analyzed for their phenolic content and antioxidant capacity, berries being among the best sources (*7*−*9*). High amounts of phenolics, particularly anthocyanins, and high antioxidant capacity are found in blackberry, black currant, and *Vaccinium* species such as blueberry, cranberry, and lingonberry (*10*−*12*). In addition to anthocyanins, chlorogenic acids (esters of *trans*-cinnamic acids, mainly caffeic and quinic acids) may provide particular health benefits by acting as strong antioxidants or directly affecting specific enzymes. Consumption of coffee beverages rich in chlorogenic acid and its derivatives has been associated with decreased risk of type 2 diabetes, the incidence of which has increased in Western societies (*13*, *14*). Chlorogenic acid content is relatively high also in some fruits, particularly in rowanberry (*15*).

Berries of rowan (*Sorbus aucuparia* L.) have been traditionally used for jellies and jams, but their wider use as food ingredients has been less popular because of their bitter taste. The first sweet rowanberry clones were selected in the Sudety mountain area, in the current Czech Republic area, in the 19th century. A breeding program for sweet rowanberries was started by Mitchurin in Russia at the beginning of the 20th century (*16*), resulting in the most interesting group of hybrids of rowanberry (*S. aucuparia*) with *Aronia*, *Malus*, *Mespilus*, or *Pyrus* species (**Table 1**). Sweet rowanberries have been bred particularly for northern conditions. The cultivars have indeed shown excellent winter-hardiness in Russia and Finland, providing thus an alternative for fruit production in these areas where the climate is unfavorable for cultivation of other fruit trees, such as pear and plum. In general, the taste of the berries is less stringent than that of rowanberries, and the berries are often larger. However, the taste and size of the berries vary greatly among cultivars (**Figure 1**). Limited data are available on the nutritional content (*17*), as well as on the antioxidant properties and phenolic content of these sweet rowanberry cultivars, which should be particularly interesting considering their diverse genetic background.

We have investigated the antioxidant properties and phenolic contents of the berries from nine sweet rowanberry cultivars, that is, Burka, Dessertnaja, Eliit, Granatnaja, Kubovaja, Rosina, Rubinovaja, Titan, and Zholtaja, and compared them with the well-characterized bilberry (*Vaccinium myrtillus* L.) and lingonberry (*Vaccinium vitis-idaea* L.). Antioxidant capacity was analyzed using two different methods, ferric reducing anitoxidant power (FRAP) and diphenylpicrylhydrazyl (DPPH), which are

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Table 1. Description of Sweet Rowanberry Cultivars Studied

cultivar	origin (discoverer, location, year, ref)	breeding background	description (berry size, color, tree height[a])
Burka	Mitchurin, Russia, 1918 (*16*, *17*)	*Sorbus aucuparia* × [*Sorbus aria* (L.) Crantz × *Aronia arbutifolia* (L.) Pers.]	medium, reddish brown, low (2–3 m)
Dessertnaja	Mitchurin, Russia, 1926 (*16*, *17*)	*Sorbus aucuparia* × *Aronia melanocarpa* L.[b] × *Mespilus germanica* L.	medium, red, low (1.5–2.5 m)
Eliit (syn. Alaja Krupnaja; Eliit 10)	Mitchurin and Tihhonova, Russia, 1926 (*17*)	*Sorbus aucuparia* × *Pyrus* sp. × *Sorbus aucuparia* var. *moravica*	very large, bright red, high (4–7 m)
Granatnaja	Mitchurin, Russia, 1925 (*16*, *17*)	*Sorbus aucuparia* × *Crataegus sanguinea* Pallas	large and angular, dark red (orange inside), medium (3–6 m)
Kubovaja	Nevezhino, Vladimir area, Russia, 19th century (*17*)	*Sorbus aucuparia*	small and elongated, orange, high (6–10 m)
Rosina	Sebnitz, Germany, 1946 (*17*)	*Sorbus aucuparia* var. *moravica*	small, reddish orange, high (6–10 m)
Rubinovaja	Mitchurin and Tihhonova, Russia, 1927 (*17*)	*Sorbus aucuparia* × *Pyrus communis* L.	medium, dark red, low (2–3 m)
Titan (syn. Titaan)	Mitchurin and Tihhonova, Russia, 1926 (*17*)	Burka × *Malus* sp./*Pyrus* sp.[c] × *Pyrus* sp.	large and angular, reddish brown, medium (3–5 m)
Zholtaja (syn. Prestnaja; Želtaja)	Nevezhino, Vladimir area, Russia, 19th century (*17*)	*Sorbus aucuparia*	small and angular, reddish orange, high (6–10 m)

[a] Tree height may vary according to the rootstock and growing conditions. [b] *Sorbus aucuparia* × *Aronia melanocarpa* = cv. Likjornaja; not analyzed in this study. [c] Mixture of pollen from *Malus* and *Pyrus* species.

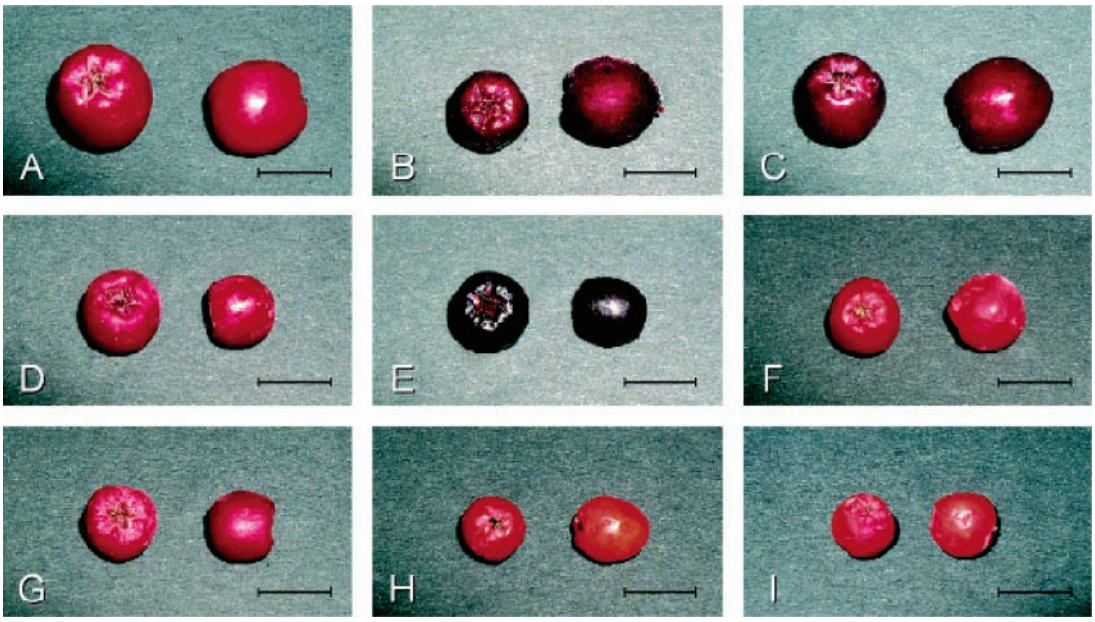

Figure 1. Berries of sweet rowanberry hybrid cultivars (**A**) Eliit, (**B**) Granatnaja, (**C**) Titan, (**D**) Dessertnaja, (**E**) Burka, and (**F**) Rubinovaja and selected *S. aucuparia* cultivars (**G**) Rosina, (**H**) Kubovaja, and (**I**) Zholtaja. Scale bars are 10 mm.

based on different end points. Phenolic contents were analyzed as total phenolics, total anthocyanins, and compound classes or individual compounds, of which chlorogenic acids were of particular interest.

MATERIALS AND METHODS

Chemicals. Acetone and formic acid of analytical grade were purchased from Fluka Riedel-de-Haën (Seelze, Germany), acetonitrile of HPLC grade was purchased from Rathburn (Walkerburn, Scotland), and Folin−Ciocalteau reagent was purchased from Merck (Darmstadt, Germany). Caffeic acid, chlorogenic acid, gallic acid, rutin, and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical were from Sigma Chemical Co. (St. Louis, MO). Cyanidin chloride was from Extrasynthese (Geney Cedex, France). 2,4,6-Tripyridyl-*s*-triazine (TPTZ) used for the FRAP assay was from Fluka Chemie AG (Deisenhofen, Switzerland). Phenolic standards were dissolved in methanol to a concentration of 1 mg/mL and stored at −20 °C.

Berry Samples and Extraction. Sweet rowanberry cultivars were obtained as grafts on rowanberry (*S. aucuparia*) rootstock from Robert Piir (Polli Experimental Station, Estonia) in 1988. Cvs. Burka, Dessertnaja, Eliit, Granatnaja, and Titan were propagated from shoot tips or axillary meristem tissues in vitro on MS medium (*18*) with 30 g/L sucrose and 1 g/L benzylaminopurine (BAP). The micropropagated plantlets were rooted and grown in the greenhouse until planted in the Research Garden of the University of Kuopio, Finland. Cvs. Kubovaja, Rosina, Rubinovaja, and Zholtaja were planted as grafts without micropropagation. Of the cvs. Dessertnaja and Granatnaja, both types (grafts, micropropagated) were grown. Ripe berries were harvested in autumn 2004 from three trees of cvs. Burka, Dessertnaja, Eliit, and Granatnaja and from one tree of cv. Titan, and in 2002 from one tree of cvs. Kubovaja, Rosina, Rubinovaja, and Zholtaja. Berry samples of cv. Granatnaja were also harvested ripe or partly ripe from three trees in 2002, as well as in 2004 from trees grown in other locations (Koijärvi, Finland; Polli, Estonia). All berries were immediately frozen and stored at −20 °C. Frozen bilberries and lingonberries were purchased from Pakkasmarja Ltd. (Suonenjoki, Finland) in 2004.

Berry samples (100 g) were crushed in a food processor. A subsample (3 g) of the homogenate was weighed and extracted three times with 15 mL of 70% acetone, pH 2, by shaking vigorously for 10 min. Supernatants were collected after centrifugation, and the three extracts were combined and filled to 50 mL with 70% acetone. The

extracts were stored at −80 °C prior to analysis. The remaining homogenate was centrifuged to separate the juice, which was used for the determination of soluble solids and titratable acids.

The percentage of soluble solids (mainly sugars) was measured as °Brix with a refractometer (Atago Co. Ltd., Tokyo, Japan). Titratable acidity was determined by titrating 1.5−5 mL of juice diluted in 100 mL of water (dilution depended on the acidity of the sample) with 0.1 M NaOH until the pH reached the value of 8.1 ± 0.2. The percentage of titratable acids is expressed as malic acid equivalents (*19*).

Phenolic Compounds. Total phenolic content was measured using the Folin−Ciocalteau method (*20*). Results are expressed as milligrams of gallic acid equivalents per gram of fresh berries. Total anthocyanin content was determined with the pH differential method (*21*), and results are expressed as milligrams of cyanidin 3-glucoside equivalents per gram of fresh berries, on the basis of the molar absorptivity (29000) and molecular mass (449.2 g/mol) of cyanidin 3-glucoside.

A high-performance liquid chromatography (HPLC) method was used for the analysis of individual phenolic compounds. Aliquots of berry extracts (1.3 mL) were evaporated to dryness in a vacuum centrifuge and dissolved in 300 μL of 50% methanol. Prior to analysis, the samples were filtered through 0.2 μm Acrodisc syringe filters (PAL Co., Ann Arbor, MI). The samples were analyzed within 2−3 h after dissolving. The HPLC system (Hewlett-Packard 1090 series, Waldbronn, Germany) consisted of two pumps, an autosampler, a column oven, and a diode array detector coupled to HP Chemstation data handling software. Samples (25 μL) were analyzed at 35 °C with a flow rate of 0.8 mL/min in a Vydac RP C_{18} column (250 × 4.6 mm, particle size = 5 μm) (The Separations Group, Hesperia, CA). Mobile phase consisted of 1% formic acid (A) and acetonitrile (B). Phenolic compounds were separated in the following gradient of B (% v/v) in A: 0−50 min, 0−15% B; 50−60 min, 15−25% B; 60−70 min, 25−100% B; 70−72 min, 100% B; 72−80 min, 100−0% B.

The phenolic compounds were classified as anthocyanins, flavonols, and hydroxycinnamic acids by their UV spectra recorded at wavelengths 520, 350, and 320 nm, respectively. Other phenolic acids, flavan-3-ols, and other unidentified compounds were omitted from this study because of their minor contribution to the total phenolic content. A number of individual compounds were identified on the basis of the literature or standard compounds run together with the samples. The phenolic compounds were quantified as equivalents of the following standard compounds: anthocyanins as cyanidin, flavonols as rutin, and hydroxycinnamic acids as caffeic acid, except for chlorogenic and neochlorogenic acids, which were quantified as chlorogenic acid. The results are expressed as milligrams of standard compound equivalents per 100 g of fresh berries.

Antioxidant Capacity. Antioxidant capacity was analyzed using two independent methods, that is, ferric reducing antioxidant power (FRAP) (*22*) and free radical scavenging capacity (EC_{50} of DPPH radical) (*23*). In the FRAP method, 30 μL of 1/40 diluted samples were analyzed as three replicates in 96-well plates with 250 μL of FRAP reagent. Changes in absorbance were recorded at 595 nm with a Labsystems Multiskan Plus absorbance reader (Eflab, Finland) after 1 h, that is, when all of the reactions had reached their steady-state values. The results are expressed as micromoles of Fe^{2+} reduced per gram of fresh berries, based on $FeSO_4$ standard analyzed in each plate.

In the DPPH method, 50 μL samples in 0.5 M sodium acetate, pH 5, were analyzed in 96-well plates with 10 mg of DPPH radical in 250 μL of methanol. Five dilutions of each sample with three replicates were analyzed to get the EC_{50} value, that is, the amount of berries needed to reduce the DPPH radical by 50%. Changes in absorbance were recorded at 530 nm in a Wallac Victor^2 1420 multilabel counter (Perkin-Elmer, Turku, Finland) every 2 min during 1 h. For each dilution, the percentage of remaining DPPH at the plateau was determined on the basis of the DPPH standard curve. The amount of berries (grams) in each dilution was plotted against the amount of DPPH radical remaining at the plateau of the reaction. Using the curve obtained, the EC_{50} value was calculated. The results are expressed as grams of fresh berries needed to reduce 1 g of DPPH by 50%. Lower values thus mean higher antioxidant capacities.

Statistical Analysis. Statistical analyses were performed with SPSS 11.5 for Windows (SPSS Inc., Chicago, IL). Total phenolic, anthocyanin, and hydroxycinnamic acid contents were plotted against antioxidant capacities, and the correlations were analyzed by calculating the Pearson correlation coefficient. The effects of ripeness, harvesting year, and storage on phenolics and on antioxidant capacity of cv. Granatnaja were tested with the analysis of variance (ANOVA). The correlations and differences were considered to be significant at $P < 0.05$. Principal component analysis (PCA) was performed to study relationships between phenolic profiles and the origin or breeding background of the sweet rowanberry cultivars. The scores for components 1 and 2 were tested with the ANOVA to detect differences between the cultivars.

RESULTS AND DISCUSSION

Phenolic Contents of Sweet Rowanberries. Total phenolic content varied greatly among the sweet rowanberry cultivars, ranging from 550 to 1014 mg/100 g of fresh weight (fw) of berries, the highest value being measured for cv. Rubinovaja (**Figure 2**). Values of 846 and 717 mg/100 g of fw were obtained for bilberry and lingonberry, respectively, being similar to those reported by others (*9*, *11*, *12*, *24*). Overall, the phenolic content was high in all sweet rowanberries compared with that of the majority of other fruits and berries, including wild rowanberry. Phenolic contents of 27−298, 38−218, and 85−130 mg/100 g of fw (*25*) and 2090 mg/g of dry weight (dw) (∼522 mg/100 g of fw) (*9*) have been reported for apple, strawberry, tomato, and wild rowanberry, respectively. Higher values have been reported for black currant, chokeberry, and crowberry (*9*, *12*), which are very dark in colored, indicating a high proportion of anthocyanins. Among sweet rowanberries, the ones with low anthocyanin content (cvs. Dessertnaja and Zholtaja) had total phenolic contents as high as in the anthocyanin-rich berries (cvs. Granatnaja, Burka, and Titan), indicating a larger proportion of other phenolics in these berries. Total anthocyanins followed the expected pattern, considerable amounts being found only in the dark red cultivars (**Figure 2**). Nevertheless, the anthocyanin content in bilberry was at least 4-fold (375 mg/100 g of fw) that in cv. Burka (80 mg/100 g of fw), which had the highest anthocyanin content among the sweet rowanberries. Our results for bilberry and lingonberry (64 mg/100 g of fw) were well in accordance with those found in the literature (*11*, *25*, *26*).

An HPLC analysis was performed to determine the relative proportions of different phenolic groups in the sweet rowanberries, bilberry, and lingonberry (**Table 2**). Hydroxycinnamic acids and flavonols constituted a significant proportion of the phenolics in sweet rowanberries. All rowanberries had qualitatively similar phenolic patterns; for example, the anthocyanin or flavonol fractions consisted of the same derivatives. However, large quantitative differences were found between cultivars.

The anthocyanin fraction in sweet rowanberries consists mainly of three compounds, which have been identified as cyanidin 3-galactoside (main fraction), cyanidin 3-glucoside (minor fraction), and cyanidin 3-arabinoside (minor fraction) (*10*). The high anthocyanin concentrations in rowanberry hybrids can be easily explained, because the same cyanidin glycosides have been found in the crossing partners (apple, pear, chokeberry, hawthorn) (*10*, *11*, *27*), elevating the anthocyanin levels naturally found in wild rowanberry. Bilberry contains one of the widest varieties of anthocyanins known within a species, but we did not pursue the identification of individual compounds of bilberry or lingonberry because extensive literature on this already exists (see, e.g., refs *10*, *26*, and *28*).

Flavonol content in the sweet rowanberries varied from 16 to 36 mg/100 g of fw (**Table 2**). The quantified fraction consisted of six compounds with typical UV spectra for

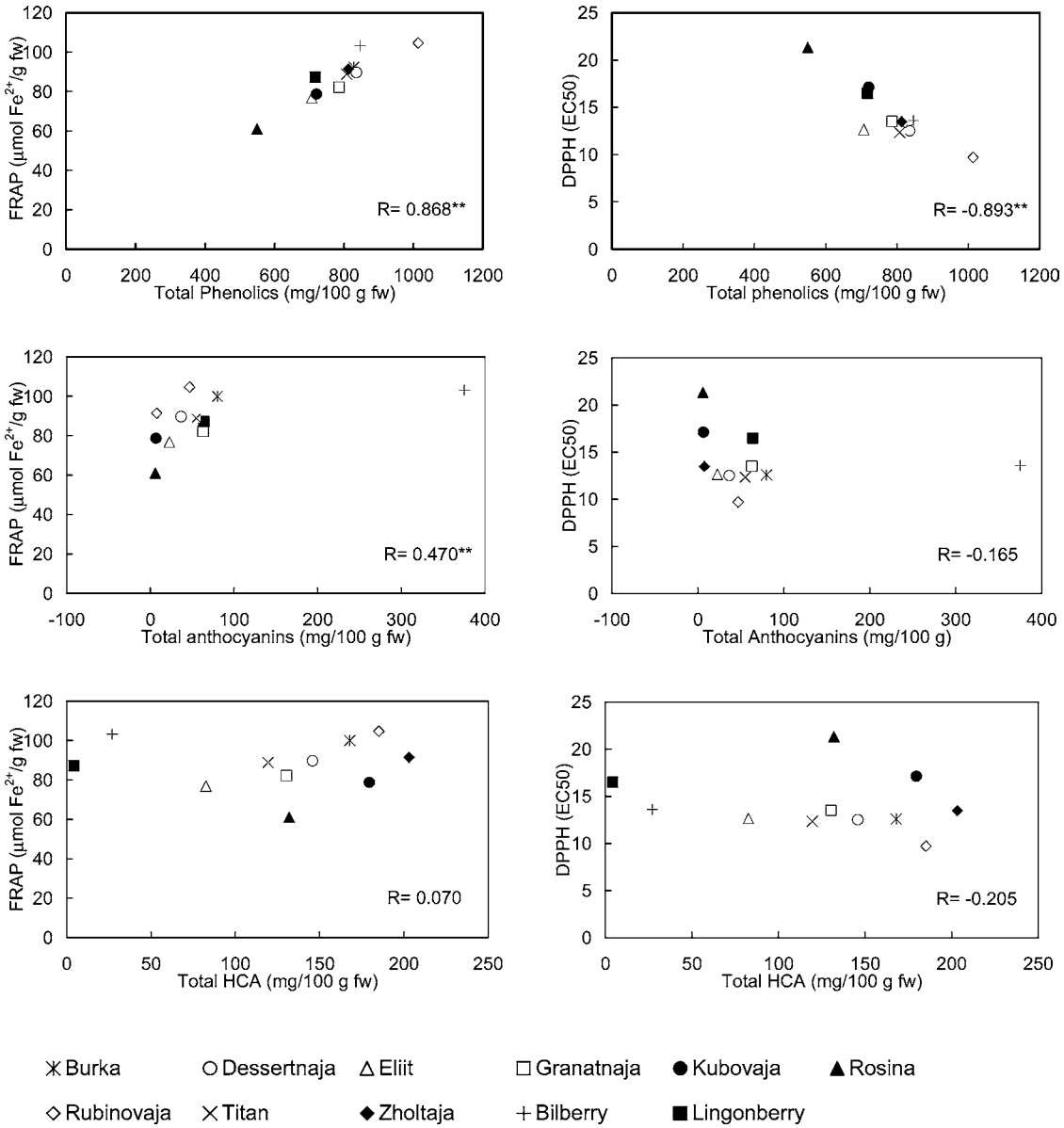

Figure 2. Correlation plots of FRAP and DPPH values versus total phenolic, total anthocyanin, and total hydroxycinnamic acid contents of sweet rowanberries, bilberry, and lingonberry. The Pearson correlation coefficients (*R*) are marked in each plot. Significant correlations are marked with asterisks ($P \leq 0.01$; two-tailed significance).

Table 2. Anthocyanins, Flavonols, and Hydroxycinnamic Acids (HCA) Quantified[a] by HPLC Based on Typical UV Spectra of the Phenolic Group

			HCA		
cultivar	anthocyanins	flavonols	chlorogenic acid	neochlorogenic acid	other HCA
Burka	156.5 ± 14.0[b]	20.2 ± 4.8	61.7 ± 6.4	103.9 ± 9.9	2.4 ± 0.18
Dessertnaja	58.9 ± 4.1	36.9 ± 6.2	84.0 ± 1.5	60.4 ± 2.4	1.5 ± 0.061
Eliit	39.5 ± 3.3	16.2 ± 5.5	29.1 ± 5.8	52.3 ± 9.7	1.2 ± 0.13
Granatnaja	116.8 ± 10.3	21.1 ± 4.1	53.4 ± 6.8	74.7 ± 8.7	2.3 ± 0.16
Kubovaja	7.0 ± 0.1	23.7 ± 0.62	138.7 ± 1.5	37.8 ± 0.57	3.0 ± 0.10
Rosina	5.7 ± 0.06	17.0 ± 0.26	94.9 ± 2.7	34.2 ± 0.51	2.9 ± 0.0072
Rubinovaja	88.9 ± 3.4	31.6 ± 2.4	108.6 ± 2.4	72.8 ± 3.0	3.7 ± 0.048
Titan	101.6 ± 4.7	18.1 ± 2.8	47.9 ± 2.3	69.2 ± 0.97	2.4 ± 0.13
Zholtaja	10.0 ± 3.6	23.7 ± 2.9	160.4 ± 2.6	39.2 ± 1.1	3.6 ± 0.18
bilberry	705.3 ± 16.3	13.8 ± 0.54	14.1 ± 1.3	nd[c]	13.0 ± 0.52
lingonberry	112.9 ± 3.6	17.4 ± 1.3	nd	nd	4.2 ± 0.21

[a] All concentrations are expressed as milligrams per 100 g of fresh berries. [b] Standard deviation (±) for the mean of three parallel samples. [c] Not detected.

flavonols, but these could not be identified on the basis of the available standard compounds or the literature. In rowanberry (*S. aucuparia*), eight flavonol compounds have been identified (*29*), of which six are quercetin and two kaempferol glycosides. Määttä-Riihinen et al. (*10*) detected quercetin derivatives (11.9 mg/100 g of fw) but only traces of kaempferol in sweet rowanberry cv. Granatnaja. The flavonol concentrations measured in the present study were higher than those reported by

Määttä-Riihinen and co-workers, possibly due to a different extraction method.

The hydroxycinnamic acids of sweet rowanberries consisted mainly of chlorogenic acid and neochlorogenic acid (**Table 2**). Neochlorogenic acid was identified on the basis of the UV spectrum and the literature (*10*, *29*). The concentrations were higher than those generally found in other berries or vegetables (*15*), which is consistent with the previous reports on sweet rowanberry (*10*) and rowanberry juice (*29*). The ratio of chlorogenic to neochlorogenic acid varied between cultivars and followed a pattern where chlorogenic acid dominated in orange rowanberry cultivars (cvs. Kubovaja, Rosina, and Zholtaja) and in cvs. Dessertnaja and Rubinovaja, whereas neochlorogenic acid dominated in the other rowanberry hybrids (**Table 2**). Chlorogenic acid has been found in all crossing partners of the sweet rowanberries (*10*, *27*−*32*), but neochlorogenic acid has been reported only for black chokeberry (*Aronia melanocarpa*) and rowanberry (*10*, *29*, *31*). No information is available on the phenolic composition of red chokeberry (*Aronia arbutifolia*), which was used in the breeding of cvs. Burka and Titan. The genetic background of sweet rowanberries, however, gives no clear explanation for the variation of chlorogenic acid ratios.

Coffee beverages have been associated with a decreased risk of type 2 diabetes, which is caused by decreased insulin secretion or insulin tolerance of beta cells. Coffee beans and, consequently, coffee beverages contain the highest measured concentrations of chlorogenic acids. A chlorogenic acid content of 280 mg/100 g of fw has been reported for Arabica coffee beans (*33*), whereas 70−200 mg of chlorogenic acids in 200 mL of coffee beverage has been analyzed (*15*). It is of particular interest that 29−160 mg/100 g of chlorogenic acid and 34−104 mg/100 g of neochlorogenic acid (isomer of chlorogenic acid) were found in sweet rowanberries, resulting in a maximum of 199 mg/100 g of total chlorogenic acids in cv. Zholtaja. Thus, the level of chlorogenic acids in rowanberries almost equals the levels found in coffee. If the positive health effects of coffee are indeed due to the high chlorogenic acid content, sweet rowanberries could be used to add a considerable proportion to the chlorogenic acid pool in the diet without the adverse effects of coffee. There is evidence to indicate that chlorogenic acid interferes with glucose transport by inhibiting glucose-6-phosphate translocase (*34*). Retardation of glucose absorption not only lowers blood glucose levels but also affects incretins, that is, gastrointestinal hormones regulating insulin secretion (*35*). Chlorogenic acid also lowers cholesterol and triacylglycerol levels in the plasma of obese rats (*36*), thus shortening free fatty acid overexposure on beta cells, which disturbs insulin secretion in obese subjects. Most of the chlorogenic acid is converted to caffeic acid or other metabolites in the gastrointestinal tract (*37*), making it difficult to determine whether the influence of coffee is due to the small amount of absorbed chlorogenic acid or to caffeic acid.

Antioxidant Capacity. Sweet rowanberries showed high antioxidant capacities, as indicated by the FRAP and DPPH methods (**Figure 2**). The FRAP value ranged from 61 to 105 μmol of Fe^{2+}/g of fw and the DPPH radical scavenging activity from 21 to 9.7 g of berries/g of DPPH radical. With both methods, cv. Rosina had the lowest and cv. Rubinovaja the highest antioxidant capacity, and these cultivars also had the lowest and highest total phenolic contents, respectively. Both antioxidant capacity assays gave similar results, the R^2 value being 0.675 for linear regression between the results from FRAP and DPPH method (data not shown). FRAP values have been measured for a large variety of fruits and vegetables (*7*). Capacities of 3.1, 6.7, 2.9, and 11.4 μmol of Fe^{2+}/g of fw have been measured for tomato, onion, apple, and orange, respectively. In berries, 24.2, 73.5, 91.7, and 395 μmol of Fe^{2+}/g of fw have been reported for wild rowanberry, black currant, crowberry, and dog rose, respectively, clearly indicating that berries are a superior source of antioxidants. Our results show that sweet rowanberries are among the berries with the highest antioxidant capacities (see Supporting Information).

There was a high correlation between the antioxidant capacity and phenolic contents (**Figure 2**). Bilberry and anthocyanin-rich rowanberry cultivars had the highest antioxidant capacities, especially with the FRAP method, but they also had the highest total phenolic contents. The correlation coefficient was lower for total anthocyanins versus antioxidant capacity than for the total phenolics versus antioxidant capacity, measured with both antioxidant methods (**Figure 2**). Bilberry, which had similar total phenolic content but at least 4-fold anthocyanin content compared with rowanberries, was not a better antioxidant source than rowanberries. Therefore, it seems that compounds other than anthocyanins contribute to the antioxidant properties of sweet rowanberries. The FRAP method showed higher correlation for anthocyanins versus antioxidant capacity ($R = 0.423$; $P = 0.004$) than the DPPH method ($R = -0.146$; $P = 0.345$), which suggests that the FRAP method gives more emphasis to anthocyanins than does the DPPH method. Indeed, the chemistries behind these two methods are somewhat different. The FRAP method is based on electron transfer assuming that the antioxidant capacity and reducing capacity are equal, whereas the DPPH method is based on hydrogen atom transfer, although Foti et al. suggest that it is also an electron transfer rather than simply a hydrogen atom reaction (*38*, *39*). Hence, some differences are likely to be observed between the results obtained with these two methods. Furthermore, the Folin−Ciocalteu method used for the total phenolic analysis is basically based on an electron transfer reaction similar to the FRAP antioxidant capacity assay, which partly explains the high correlation generally found between total phenolic contents and antioxidant capacities (*38*).

Because chlorogenic acids constitute a significant fraction of phenolics in sweet rowanberries, we also studied the possible correlation between antioxidant capacities and hydroxycinnamic acid contents. As shown in **Figure 2**, no general correlation exists.

Rowanberries contain compounds other than phenolics, such as carotenoids, vitamin E, and vitamin C, which might contribute to the antioxidant capacity. According to Piir and Niiberg (*17*), carotenoid levels in sweet rowanberries are as high as those in carrots, and vitamin C levels are close to those of strawberries, varying from 21 (Granatnaja) to 86 mg/100 g (Zholtaja). More vitamin C is found in rowanberry varieties (*S. aucuparia*) than in the hybrid cultivars. Vitamin C and carotenoids cannot be efficiently extracted with the method used in this study, and it is thus unlikely that they have significantly affected the antioxidant capacities. Furthermore, the correlation between antioxidant capacity and phenolics was so evident that the high capacities observed are probably due to the high phenolic content of the samples.

Ripeness and Storage. Because berries from two different growing seasons (2002 and 2004) were used, and some of them after a fairly long storage time, a comparative analysis was performed for cv. Granatnaja, for which ripe berries from both years were available from three locations in 2004. For this cultivar, moderately colored yet unripe berries from 2002 were also analyzed. The 2002 berries were analyzed twice, after 1

Table 3. Variation in Phenolic and Anthocyanin Contents and Antioxidant Capacities within Cv. Granatnaja Berries Differing in Ripeness, Harvesting Year, or Location

tree[a]	soluble solids (°Brix)	titratable acids (%)	ripeness index (°Brix/acids)	total phenolics (mg/100 g)	DPPH EC_{50}
Year 2002, Unripe (a) and Ripe (b) Samples from Kuopio, Analyzed in 2003[b]					
1a	13.8	2.2	6.3	839	10.4
1b	18.8	1.7	11.1	829	12.3
2a	14.2	2.3	6.2	792	13.2
2b	19.4	1.8	10.8	838	12.6
3a	14.5	1.8	8.1	887	11.1
3b	17.9	1.2	14.9	790	11.9
Year 2002, Ripe Samples from Kuopio, Analyzed in 2004					
1	19.0	1.7	11.2	958	10.8
3	18.8	1.6	11.8	926	10.3
4	19.6	1.7	11.5	916	10.6
Year 2004, Ripe Samples from Kuopio, Polli, and Koijärvi, Analyzed in 2004					
1	12.2	1.7	7.2	798	13.4
2	13.4	1.7	7.9	826	12.9
5	12.2	1.8	6.8	733	14.2
Polli	12.3	2.1	5.9	913	10.9
Koijärvi	12.1	1.4	8.6	869	12.0

[a] Trees are numbered similarly in Kuopio in every year. [b] Time between harvesting of unripe and ripe samples was 4 weeks.

Table 4. Soluble Solids and Titratable Acids in Berries of Sweet Rowanberry Cultivars, Bilberry, and Lingonberry

cultivar	°Brix [a]	acidity[b] (%)	°Brix/acids
Burka	11.3	1.4	8.1
Dessertnaja	15.4	1.6	9.7
Eliit	12.8	2.5	5.1
Granatnaja	12.6	1.7	7.4
Kubovaja	21.5	3.4	6.3
Rosina	19.1	3.0	6.4
Rubinovaja	16.1	1.9	8.6
Titan	11.9	1.6	7.7
Zholtaja	19.8	3.0	6.6
bilberry	9.7	1.2	8.3
lingonberry	11.2	4.6	4.6

[a] Soluble solids are expressed as °Brix (% w/v). [b] Titratable acids are expressed as malic acid equivalents (% w/v).

and 2 years of storage. Results for total phenolics, DPPH antioxidant capacity, soluble solids and titratable acids are shown in **Table 3**. **Table 4** displays the soluble solids and titratable acids for all rowanberry cultivars, bilberry, and lingonberry. The ratio of soluble solids (sugars) to titratable acids is species- and cultivar-specific, but it can be also used as an indicator for ripeness stage within a cultivar. Partly unripe berries of cv. Granatnaja have a ripeness index of ~6.0, whereas ripe berries have an index of >10 (**Table 3**). Thus, in 2004, the berries had been harvested before they were fully ripe, possibly due to exceptionally unfavorable weather conditions during the growing season. However, the ripeness stage, varying from partly ripe to ripe (in 2002 samples), seems not to influence the total phenolic content or antioxidant capacity ($P > 0.05$). Equally, the harvesting year did not affect total phenolics and antioxidant capacity significantly. Only slight variation has been reported in the phenolic contents between different ripeness stages in lingonberry (*24*) and in sweet cherry (*40*), which supports our results. However, in berries in which the phenolic fraction consists mostly of anthocyanins synthesized at the late stages of ripening (e.g., blueberry), greater changes can be found

Table 5. Phenolic Compounds Divided into Four Principal Components

compound	component	factor loading	% of variance explained
cyanidin 3-galactoside	1	0.930	39.5
cyanidin 3-glucoside	1	0.779	
cyanidin 3-arabinoside	1	0.904	
chlorogenic acid	1	0.703	
neochlorogenic acid	1	0.921	
flavonol 3[a]	1	0.725	
flavonol 1	2	0.775	24.0
flavonol 2	2	0.871	
flavonol 4	3	0.494	14.2
flavonol 6	3	0.724	
flavonol 5	4	0.742	9.2

[a] Flavonols are numbered in the order of their elution in HPLC.

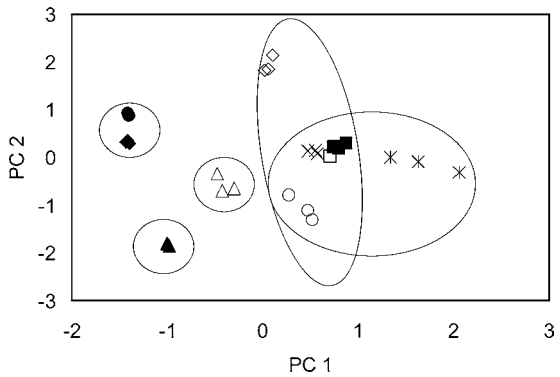

Figure 3. Scatter plot of regression factor scores of components 1 and 2 obtained for sweet rowanberry cultivars. The circles separate clusters of cultivars significantly different (ANOVA; $P < 0.05$) in component 1. Three parallel berry samples were analyzed for each cultivar.

(*41*). Differences between individual trees or locations were not statistically analyzed because of the limited data available, but in these specific samples the data obtained from different trees and locations were similar to each other.

The levels of total phenolics were slightly higher ($P = 0.068$) after 2 years of storage than after 1 year, when samples from the same trees were examined (**Table 3**). Similarly, the antioxidant capacities were higher ($P = 0.026$) after longer periods of storage. Changes in phenolic contents under different storage conditions and times have been studied more extensively with other berries. Generally, it has been concluded that phenolic contents and, therefore, antioxidant capacities can increase during storage, even at low temperatures (*40*, *41*). However, the increase observed in our study is not high enough to cover the differences between the cultivars, even though the specific values obtained for cultivars harvested in 2002 should be subjected to certain reservations when compared with the values obtained for cultivars harvested in 2004.

Principal Component Analysis. PCA was performed to classify sweet rowanberry cultivars on the basis of their phenolic composition (anthocyanins, chlorogenic acids, and flavonols). **Table 5** shows the compounds analyzed and their factor loadings in four principal components (PC1−4), which explain 86.9% of the total variation of the data set. The first component is composed of anthocyanins and chlorogenic acids, whereas the other three components (PC1−3) are composed of flavonols. **Figure 3** shows the projection of rowanberry cultivars on the PC1 and PC2 plane, based on the regression factor score values obtained for each cultivar. The cultivars were efficiently separated on those two planes, forming clusters of cultivars, of

which significantly different in PC1 are marked with circles in **Figure 3**. In PC2, cvs. Rubinovaja, Kubovaja, and Rosina were significantly different from the other cultivars. In general, cultivars were clustered in the plot strongly based on the color of berries (**Figure 1**), the orange cultivars (*S. aucuparia* clones) being located on the left side and the dark red cultivars on the right side. Cv. Eliit, which is a double-cross of *S. aucuparia* (**Table 1**), is also located closest to the true *S. aucuparia* cultivars in the plot. Interestingly, the German cv. Rosina was significantly different in both PC1 and PC2 from the Russian cvs. Kubovaja and Zholtaja, although they all are clones of *S. aucuparia* (**Table 1**). Furthermore, cvs. Granatnaja and Titan were very similar, forming a single group in PC1 and PC2, which is supported by the fact that the appearance of the berries from those cultivars is almost identical and the main difference between the cultivars is the growth form of the trees. In cv. Granatnaja, the data from 2002 and 2004 were similar, giving a single group in PC1 and PC2. The PCA was also performed with four unidentified phenolic acids, which were excluded from the quantitative analysis, but the clusters of cultivars were not affected despite the presence of additional compounds in the analysis.

In summary, sweet rowanberries were shown to contain high concentrations of phenolics, namely, chlorogenic acids, anthocyanins, and flavonols, the concentrations of which were cultivar-specific. They also possess a high antioxidant capacity, which was confirmed with two different methods. Considering the excellent cultivation and antioxidant properties of sweet rowanberry cultivars, they could find use as good sources of cultivated berries for industrial use, especially in northern areas.

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Supporting Information Available: Table on total phenolic, total anthocyanin, FRAP, and DPPH values for sweet rowanberries, bilberry, and lingonberry. This material is available free of charge via the Internet at http://pubs.acs.org.

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